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ORIGINAL ARTICLE

Emergence of Tigecycline Resistance among Extended Spectrum Beta Lactamases Producing Gram-Negative Organisms

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ABSTRACT

Objective: To highlight the emergence of Tigecycline resistance among ESBL producer gram negative organisms.

Patients and Methods: This cross-sectional study was conducted in Lahore College for Women University Lahore Pakistan from 1st October 2015 to 20th March 2016. A total of 360 isolates of gram-negative rods were selected and processed for identification of ESBL producers via double disc synergy technique. In total 100 isolates were identified as ESBL producing gram-negative rods (*Escherichia coli, Klebsiella* spp., *Proteus* spp. and *Acinetobacter spp.)* and were further processed for antimicrobial resistance testing against Tigecycline disks (30ug) via Kirby Bauer Disc Diffusion method.

Results: Out of 360 identified gram-negative rods, 100 (27.7%) were ESBL producers. Among these total 46% samples were from male patients and 54% from female patients. A maximum number of ESBL producers were recovered from pus & wound samples (54%). The most common ESBL producer was *Escherichia coli* followed by *Klebsiella* spp., *Proteus* spp. and *Acinetobacter spp.* Overall susceptibility rate of Tigecycline was 54%. Tigecycline resistance was greatest for ESBL producing Acinetobacter (n=8), followed by Proteus (n=14), Escherichia coli (n=18) and Klebsiella (n=6) (p=0.004).

Conclusion: Although tigecycline showed very good results against ESBL producers, emergence of Tigecycline resistant ESBL producers is an alarming situation.

Key words: Drug-resistance, Extended Spectrum Beta Lactamase, Tigecycline.

Author`s Contribution
[1] Conception, synthesis, planning of research and manuscript writing Interpretation and discussion
[2,3] Data analysis, interpretation and manuscript writing, [4] Active participation in data collection.

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Article info.
Received: March 10, 2018
Accepted: May 14, 2018

Cite this article. *Saleem NM, Saleem N, Hafeez A, Shaheen S. Emergence of Tigecycline Resistance among Extended Spectrum Beta Lactamases Producing Gram-Nagitive Organisms. JIMDC.2018; 7(2):118-122*

Funding Source: *Nil*
Conflict of Interest: *Nil*

Introduction

Infections caused by gram-negative bacteria have continued to be a major problem, especially in hospitalized patients. These are responsible for serious bloodstream infections, respiratory tract, skin and skin structure infections. Carbapenemases are a major cause of carbapenem resistance in Enterobacteriaceae.[1] Extended-Spectrum Beta-Lactamases are mainly produced by the *Enterobacteriaceae* family (and non-fermentative gram-negative organisms) and are capable of hydrolyzing extended-spectrum beta-lactam antibiotics

like cephalosporin, monobactam and penicillin.[2,3] The efforts to treat multidrug-resistant (MDR) microorganisms are mainly focused on gram-positive bacteria, evident by the development of novel antimicrobial agents like linezolid.[4] Infections caused by MDR gram-negative bacilli have become a growing problem. Resistance to fluoroquinolones, co-trimoxazole and trimethoprim is frequently observed among ESBL producers, resulting in treatment failure and also greatly limiting the choice of antibiotics that can be used for treatment.[5]

Carbapenems have been important drugs for the treatment of infections caused by ESBL producing *E. coli* and *K. pneumoniae*, however, carbapenem resistance is emerging in many areas of the world.[6-8] Tetracycline derivative, Tigecycline has proved a remarkable drug, having in vitro activity against many of these MDR organisms.[9] Tigecycline is the first in a new class of antibiotics, the glycylcyclines, licensed by the US Food and Drug Administration (FDA) in June 2005 for intravenous use in adults. Protein synthesis is ultimately inhibited, thereby exerting a bacteriostatic effect.[10] Tigecycline exhibits potent in vitro activity when tested against a broad spectrum of both tetracycline-susceptible and tetracycline-resistant gram-positive and gram-negative bacteria. There is a lack of data on the treatment of severely ill patients from the pivotal trials with Tigecycline. Available data on the use of Tigecycline in severely ill patients are mostly from retrospective analyses of studies with a focus on identified pathogens rather than the clinical picture.[11] This study aimed at determining the resistance rate of ESBL producing bacteria to Tigecycline. As the prevalence of ESBL-producing bacteria is on the rise, newer choices of antibiotics for such organisms will help in treating critically ill patients infected by them.

Patients and Methods

This cross-sectional study was conducted in Government College for Women under Lahore College for Women University Lahore Pakistan during the period of 1st October 2015 to 20th March 2016. Standard microbiological procedures like Gram staining, colony morphology, biochemical tests and Analytical Profile Index (API) were applied to differentiate the strains of gram-negative bacteria. Total 360 isolates of gram-negative rods were selected from tertiary care hospital Lahore by using convenient sampling technique. These isolates were processed for the identification of ESBL producers via Double disc synergy technique. Then ESBL producing gram-negative rods were further processed for antimicrobial resistance testing against Tigecycline disks (30ug) via Kirby Bauer Disc Diffusion method. Tigecycline susceptibility of different ESBL-producing gram-negative rods was compared by Pearson's chi-square. Their respective inhibition zone diameters were compared by ANOVA, keeping a value of $p \leq 0.05$ to be statistically significant.

Results

Out of 360 identified gram-negative rods, 100 (27.7%) isolates were ESBL producers. Out of 100 ESBL producing gram-negative rods, 46% samples were from males and 54% from female patients. Regarding sample-wise distribution, ESBL producers were most commonly found in pus & wound (54%), followed by urine samples (22%) (Figure 1). The most common ESBL producer was Escherichia coli 50% (n=50) followed by Klebsiella spp. 20% (n=20), Proteus spp. 20% (20%) and Acinetobacter spp 10% (n=10). Overall susceptibility rate of tigecycline was 54% (Figure 2).

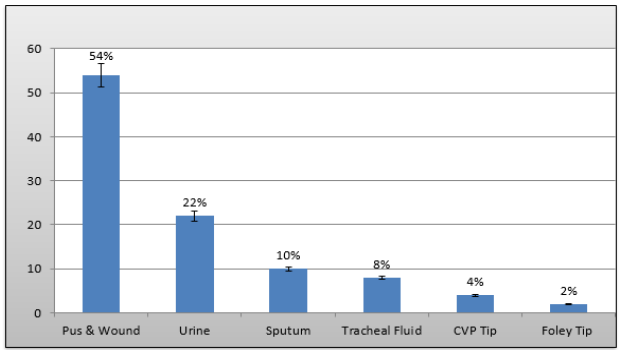

Figure1: Sample wise isolation rate of ESBL producers.

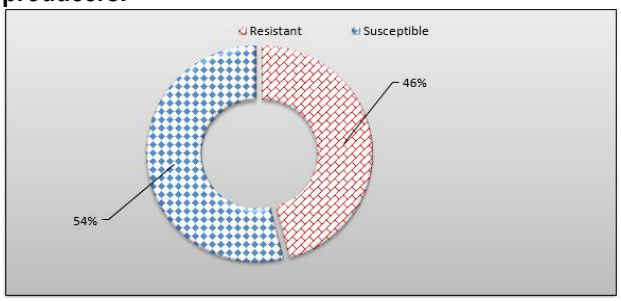

Figure 2: Overall Tigecycline resistance rate.

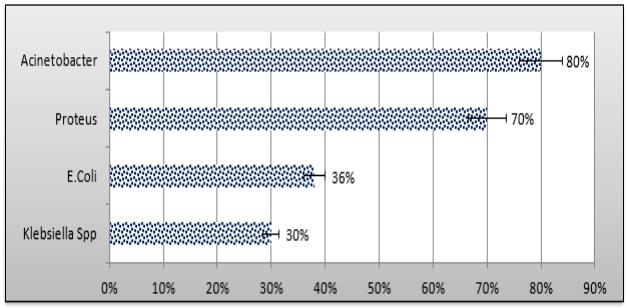

Figure 3: Organisms wise resistance pattern to Tigecycline.

Tigecycline resistance rate was significantly different among four strains (p=0.004). Resistance was greatest for ESBL producing Acinetobacter (80% [n=8]), followed by Proteus ([70%] n=14), Escherichia coli (36%/ n=18) and Klebsiella (30%/n=6) (Figure 3). The mean clearance zone diameter was 19 ± 2 mm (95% CIs 18.4, 19.6). The four strains did not differ significantly in terms of mean inhibition zone diameters (p = 0.827) (Table 1).

Table 1: Inhibition zone diameter of four strains

Strains	Inhibition zone diameter mean±SD	p-value
Escherichia coli (mm)	19±2	0.274
Klebsiella spp (mm)	19±1	
Proteus spp (mm)	18±1	
Acinetobacter spp (mm)	19±2	

Discussion

There are serious concerns regarding increasing trend of antimicrobial resistance among gram-negative bacteria as there has been little successful development of new antimicrobial agents targeting this group of organisms. Keeping in view this increasing burden of multi-drug resistant gram-negative rods and limited treatment options available, our study aimed to determine Tigecycline susceptibility in one major subgroup responsible for multi-drug resistance among gram-negative rods, i.e. ESBL-producing gram-negative rods, to widen the treatment options available for treating infections with such organisms and to prevent treatment failure. In a recent report, the Infectious Diseases Society of America specifically addressed three categories of MDR gram-negative bacilli, namely, extended-spectrum cephalosporin-resistant *E. coli* and *Klebsiella spp.,* MDR *P. aeruginosa* and carbapenem-resistant *Acinetobacter spp.*[12] Contrary to what happened with gram-positive bacteria, no antibiotic from a new class has been developed specifically for MDR gram-negative bacilli. The glycylcyclines, Tigecycline is an exception, although was not developed specifically for the purpose of treating infections caused by such bacteria, this drug was found to have effective in-vitro activity against many of the MDR gram-negative bacilli.[13]

In our study, 80% of the *Acinetobacter* strains were resistant to Tigecycline. This finding is slightly higher than observed by Navon-Venezia *et al* [6], reporting 78% of the multidrug-resistant *A. baumannii* isolates were resistant to Tigecycline. It is in complete agreement with Liu *et al.*, a study that compared the in vitro activity of Tigecycline against 3,014 isolates of clinically important drug-resistant bacteria using disk diffusion methods, they included ESBL -producing *E. coli* (n = 602), *K. pneumoniae* (n = 736) and also *A. baumannii* (n = 726) that had been collected from patients, treated between 2008 and 2010 at 20 hospitals in Taiwan. They suggested Tigecycline resistance rate of 30.1% in the disk diffusion testing method found among the ESBL-producing *K. pneumoniae* isolates.[14]

In present study *E. coli* showed the highest rate of ESBL production which is similar to the study of Pallett and Hand[15], in which it was concluded that CTX-M-producing *E. coli* often occurs in the community and *E. coli* is one of the commonest organisms causing urinary tract infections (UTIs) the choice of agents to treat these infections is diminishing. In the setting of infections by multi-drug resistant organisms like ESBL producers, Tigecycline remains our most reliable resort among all extended-spectrum antimicrobials. Nandi et al reported out of 82 E.coli, 14 (17.0%) were ESBL producers, none of them showed resistance to Tigecycline and out of 67 Klebsiella isolates, 21 (i.e 31.34%) were ESBL producers out of which only 1 (4.7%) was resistant to Tigecycline. Out of 19 Acinetobacter spp isolated 3 (15.7%) were resistant to Tigecycline.[16] Gill et al reported that 56.4% of the isolates were Escherichia coli, 28.2% were Klebsiella pneumoniae, 10.26% were Enterobacter species, and 2.6% were each Klebsiella oxytoca and Acinetobacter species. ESBLs were found to be most sensitive to

tigecycline, intermediate in susceptibility to minocycline while least sensitive to doxycycline and tetracycline.[17] Khalid et al reported that out of 826 clinical isolates of Gram negative bacilli, 364 were ESBL producers. Escherichia coli was the most frequent ESBL producer followed by Klebsiella pneumoniae and Enterobacter spp. Carbapenems were found to be the most effective drug followed Tigecycline then Amikacin and Nitrofurantoin.[18] Naz et al reported 100% resistance in MβL positive isolates for Imipenem, Piperacillin + Tazobactum, Ceftriaxone, Co-amoxyclav, Cefoperazone+Sulbactam, Ciprofloxacin, and Amikacin, Doxycycline, and Gentamicin showed 91.2%, 94.0%, and 97.5% resistant rate respectively. No resistance was observed against Colistin.[1] Although the research is going on in developing newer antimicrobial agents, yet their rate of development is quite slow.[19] Hence, judicious use of antibiotics and appropriate antiseptic measures are the prime requirements in order to curtail the ever increasing resistance.[19] The broad-spectrum antibiotics should be used empirically only in the serious infections and when the facility for susceptibility testing is not available.[19] The authors further recommend a large scale in vivo study in order to establish the in vivo efficacy of Tigecycline against ESBL producing gram-negative rods.

Conclusion

Although tigecycline showed very good results against ESBL producers, however, emergence of Tigecycline resistant ESBL producers is an alarming situation.

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